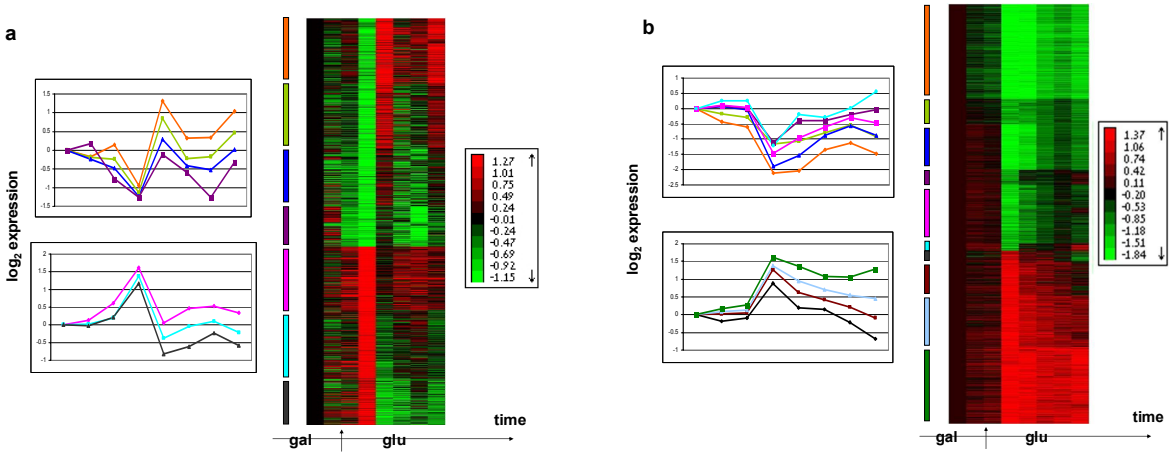
Genome-wide transcriptional plasticity underlies cellular adaptation to novel challenge/ Stern et al.

Supporting figures and tables:



c

40mM	transiently induced	transiently repressed	other kinetics	total
no3AT				
transiently induced	170	31	342	543
transiently repressed	103	219	370	692
other kinetics	428	748	1737	2913
total	701	998	2449	4148

Fig. S1: Clustering analysis. Only active genes which exhibited at least a two fold change in at least one time point along each of the experiments were subjected to clustering (2224 and 2350 genes for the no-*3AT* and 40 mM *3AT* experiments, respectively, out of 4148). The Self Organizing Maps (SOM) clustering method(Tamayo et al., 1999) which is implemented in the EXPANDER microarray analysis package(Shamir et al., 2005) was applied to these gene profiles, with 16 clusters as a pre-defined parameter (the results are not sensitive to the predefined number of clusters parameter implemented in the SOM algorithm). The 16 clusters (overall average homogeneity: 0.77 and 0.88 for the no *3AT* and 40 mM *3AT*, respectively) show that ~55% (1235) of the active genes for the no-*3AT* experiments and ~72 % (1699) of the active genes for the 40 mM *3AT* experiment, assigned to 7 clusters in the former and 10 clusters in the latter, exhibited a mean expression pattern of significant induction/repression (>2 fold change) upon switching to glucose and then a relaxation on the time scale of cells adaptation. (a) Color coded expression

levels for the 1235 genes of the no-*3AT* experiment and the associated time course of their mean values in 7 clusters exhibiting similar dynamics (each trajectory on the left corresponds to a cluster of genes marked by the same color bar on the right). (b) The same as in (a) for the 40 mM *3AT* experiment, for the 1699 genes and the associated 10 clusters. These clusters which exhibited the same mean expression profiles were joined to form the two dominant global clusters depicted in Fig. 1 (main text). The average homogeneity of the global clusters was 0.78 for the no-*3AT* experiment and 0.85 for the 40 mM *3AT* experiment.

(c) **Clusters comparison between the experiments.**

The amount of overlap between the global clusters in the two experiments, with and without 3AT (Fig. 1, main text) was computed. 31% of all genes in the no-*3AT* transiently induced cluster were also present in the transiently induced cluster of the *3AT* experiment (170 genes). 5 % of these genes were present in the transiently repressed cluster of the *3AT* experiment (31 genes) and 64% of genes exhibited other dynamics in the *3AT* experiments (342 genes). 31% of all genes in the no-*3AT* transiently repressed cluster were also present in the transiently repressed cluster of the *3AT* experiment (219 genes). 15 % of these genes were present in the transiently induced cluster of the *3AT* experiment (103 genes) and 54% of genes exhibited other dynamics in the *3AT* experiments (370 genes).

Interestingly, 26% of all genes exhibiting other dynamics in the no-*3AT* experiment were present in the transiently repressed cluster of the *3AT* experiment (748 genes), 15% of these genes were present in the transiently induced cluster of the *3AT* experiment (428 genes) and 59% continued to show other dynamics.

The total fraction of overlap between the two global clusters in the two experiments was only partial (15%), but significantly larger than would expected by chance (*chi*-test, $p<10^{-37}$).

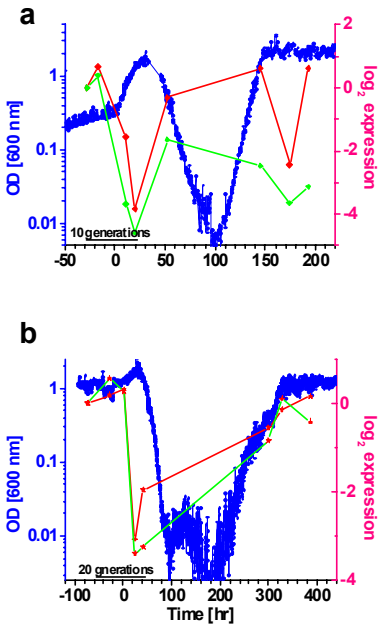


Fig. S2: Expression profiles of the GAL genes. The expression profiles of *GAL1* (red) and *GAL10* (green) genes superimposed on the population density curves (blue line) along the two experiments (a) no-*3AT*, and (b) 40 mM *3AT*. In agreement with k-PCR results in ref (Stolovicki et al., 2006) . Note the logarithmic scale.

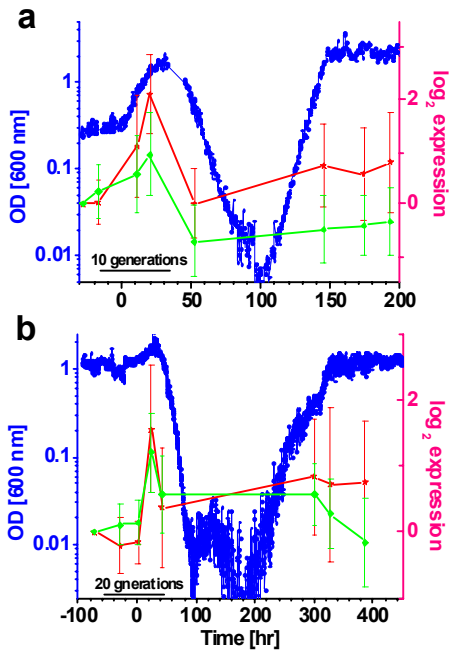
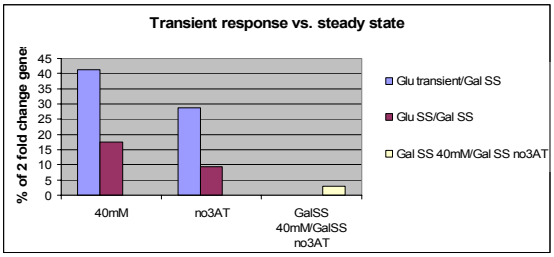


Fig. S3: Expression profiles of ribosomal and glycolysis genes. The mean expression levels as extracted from the array data for ribosomal protein genes (98, green) and glycolysis genes (14, red) superimposed on the population density curves (blue lines) along the two experiments (a) no-*3AT*, and (b) 40 mM *3AT*. The data points are average over all the genes belonging to these functional modules as defined in *GO*(www.yeastgenome.org) and the error bars present the standard deviation of expression values among genes belonging to each module. Note the logarithmic scale.

b



number of genes

	transient	SS
no 3AT induced	564	112
no 3AT repressed	625	274
40mM 3AT induced	667	257
40mM 3AT repressed	1045	470
total:	4148	

Fig. S4: Comparison between the transients and steady states of the two experiments, **with and without *3AT***. Plotted are the fractions of genes which exhibited at least 2 fold expression changes between: (i) the transient response in glucose (4^{th} time point) and galactose steady state, (ii) glucose steady state and galactose steady state, and (iii) galactose steady states in the two experiments. The table presents the actual number of genes for each case. Note the similarity in transcriptional state for the galactose steady state in the two experiments.

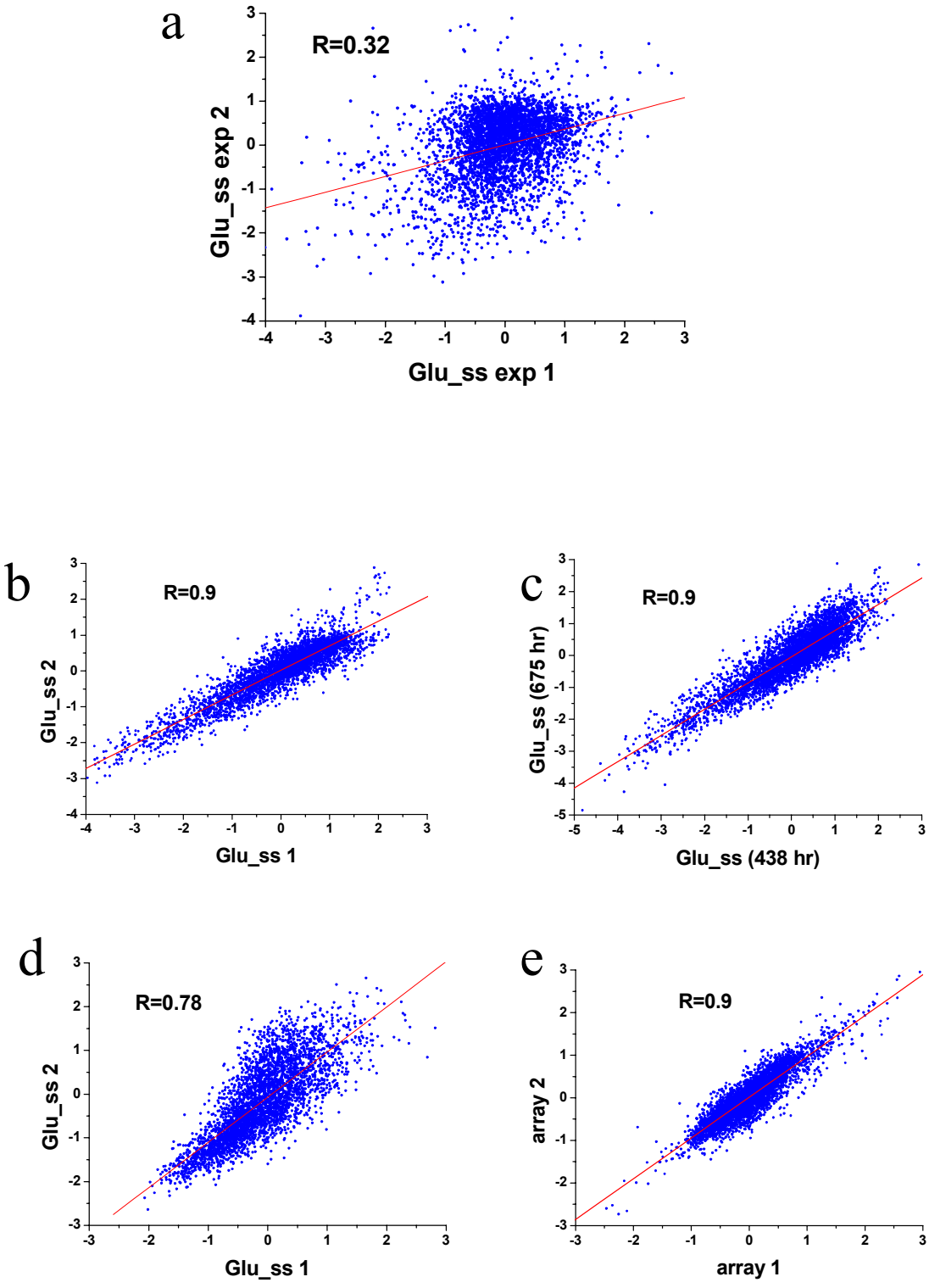


Fig. S5: Comparison between the transcriptional glucose steady states of two repeated experiments.

The no 3AT chemostat experiment was repeated with the same parameters as in the first experiment. The chemostat was switched from galactose to glucose containing medium exactly as in the first experiment and samples were collected at time points along the experiment. The chemostat dynamics (OD) showed the same phases and the same steady state in glucose as in the first experiment. We analyzed the mRNA expression using the same type of arrays and the same reference as in the first experiment. The figures show the expression values (in log2 scale) for the genes that passed all filters in the no *3AT* experiments and the 40 mM *3AT* experiment discussed in the main text (3728 genes).

a. Comparison between the glucose steady states of the two repeated experiments (exp1 is the one discussed in the main text). b. Comparison between two time points separated by 16 hrs within the glucose steady state of the same no *3AT* experiment. c. Comparison between two time points within the glucose steady state of the same no *3AT* experiment, separated by 237 hrs. This result shows the stability of the glucose steady state expression pattern. d. Comparison between two time points within the glucose steady state of the 40 mM *3AT* experiment, and e. as a reference the figure shows a comparison of typical array duplicates at the same point reflecting the error in the array experiments. The red lines are linear fits to the data and the R values are the Pearson correlation coefficients between the experiments on the two axes. Note the significantly lower correlation for separate experiments compared to time points within the same experiment.

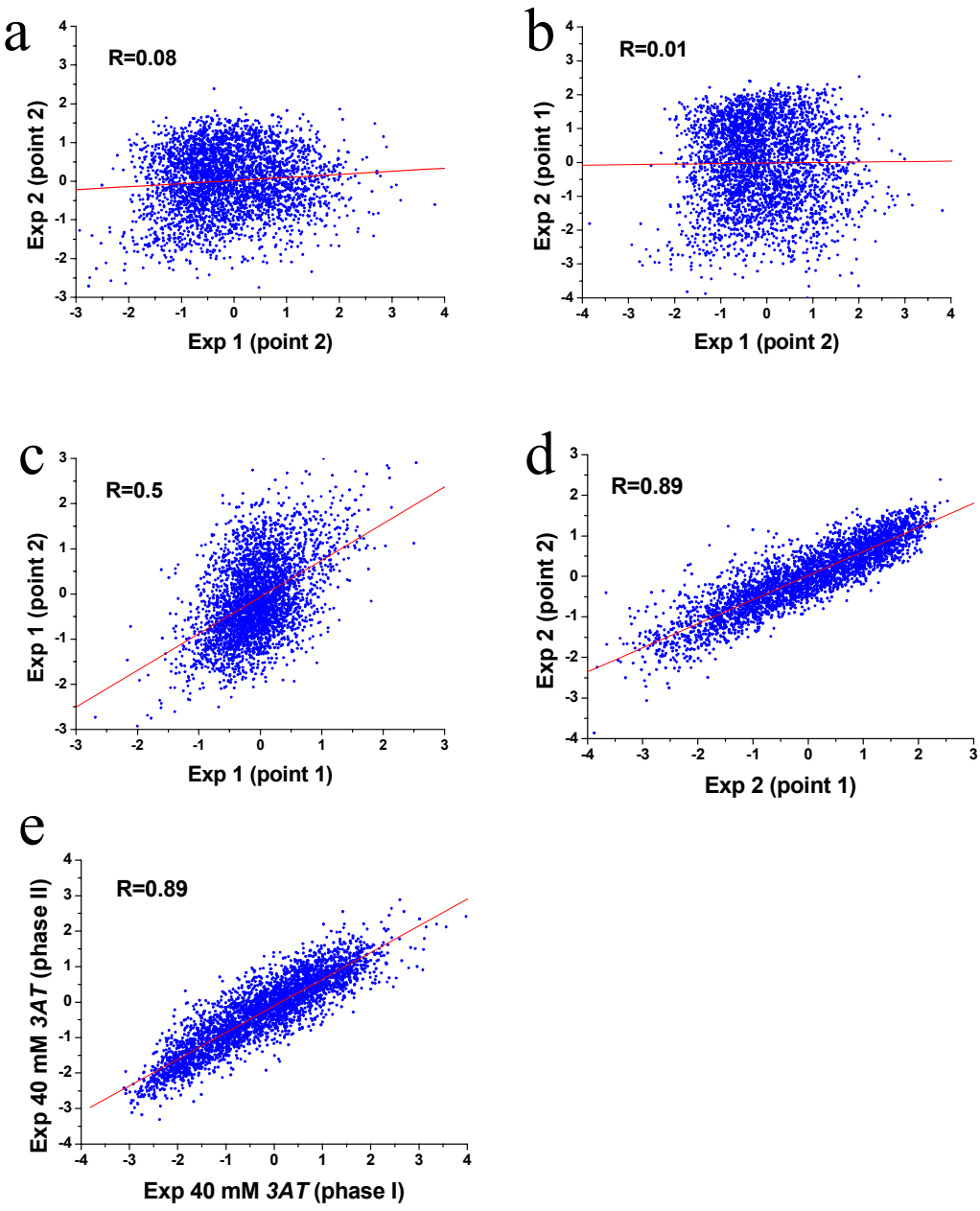


Fig. S6: Comparison between the transcriptional patterns of transient responses following the switch into glucose medium in two repeated experiments.

The same repetition experiment as in Fig. S5. The figures show the expression values (in log2 scale) for the genes that passed all filters in the no *3AT* experiments and the 40 mM *3AT* experiment discussed in the main text (3344 genes).

a. Comparison between two transient points in phase I of the population dynamics (see Fig. 1 main text) for the two repeated experiments with no *3AT*. b. Comparison between two other transient points in phase I of the population dynamics for repeated experiments. c. Two transient time points within the phase I dynamics of the first no *3AT* experiment, d. the same as c for the second no *3AT* experiment and e. comparison between two transient points in phase I and phase II of the dynamics for the 40 mM *3AT* as described in the main text. The red lines are linear fits to the data and the R values are the Pearson correlation coefficients between the experiments on the two axes. Note the significantly lower correlations between two repeated experiments compared to time points within the same experiment.

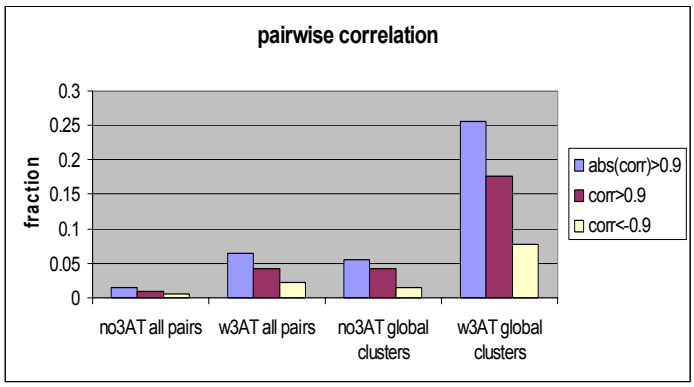


Fig. S7: Comparing the correlations of transcriptional response in the two experiments. A Pearson correlation coefficient was computed between all pairs of genes in both experiments, with and without *3AT*. The fraction of gene pairs which exhibited high positive or negative correlation (>0.9 or <-0.9) in the 40mM *3AT* experiment (6.5%) was significantly higher than in the no-3AT experiment (1.5%). The figure also presents the pair-wise correlation between genes assigned to the global clusters of Fig. 1 (main text). The fraction of gene pairs which exhibited high positive or negative correlation (>0.9 or <-0.9) in the 40mM *3AT* experiment (25%) was significantly higher than in the no-3AT experiment (5%).

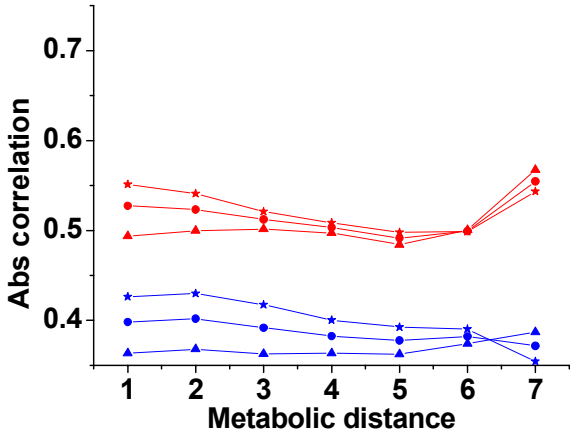


Fig. S8: The correlation coefficient between pairs of metabolic enzymes versus their metabolic distance. The metabolic network was constructed according to ref (Kharchenko et al., 2005). Following their analysis, the shortest path (number of metabolites) was computed between all pairs of enzymes. The Pearson correlation coefficient was computed for each pair of enzymes using their transcriptional dynamic profiles in each experiment (blue-no *3AT*, red 40 mM *3AT*). The mean absolute correlation coefficient of all pairs within a bin of given metabolic distance (with a width of one metabolic distance) is plotted against the metabolic distance (circles-all correlations, triangles-negative correlations and stars-positive correlations). The average metabolic distance of the network is ~3.5.

Table S1: Comparison to environmental stress response

general stress response
induced genes

transitions	fraction no3AT	fraction 40mM 3AT	# genes no3AT	# genes 40mM 3AT	
a (induced)	0.07960199	0	16	0	out of 201
a (repressed)	0.024875622	0.024875622	5	5	
b (induced)	0.194029851	0.154228856	39	31	
b (repressed)	0.084577114	0.248756219	17	50	
c (induced)	0.174129353	0.039800995	35	8	
c (repressed)	0.189054726	0.034825871	38	7	

repressed

transitions	fraction no3AT	fraction 40mM 3AT	# genes no3AT	# genes 40mM 3AT	
a (induced)	0.0375	0	12	0	out of 320
a (repressed)	0.01875	0.009375	6	3	
b (induced)	0.16875	0.296875	54	95	
b (repressed)	0.084375	0.15	27	48	
c (induced)	0.196875	0.003125	63	1	
c (repressed)	0.265625	0.065625	85	21	

The fraction of genes which showed at least 2 fold induced/repressed expression change compared to the galactose steady state in our experiments and were also induced or repressed in the environmental stress response (ESR, Fig. 3 in ref (Gasch et al., 2000)) was computed: (a) the first time point in glucose (3rd time point, phase I in Fig. 1 main text) (b) the peak of the transient response (4th time points, phase I in Fig. 1 main text) relative to the galactose steady state and (c) the first relaxation point (5th time point, phase II in Fig. 1 main text) relative to the 4th point. Choosing this latter reference point eliminates the effect of the galactose to glucose transition. We excluded the genes of the ribosomal proteins from the repressed ESR cluster to reduce a possible bias toward one large coherent group of genes, although it does not significantly change the results. It is clear that the overlap between the expression patterns observed in our experiments and that of the known stress response is very small.

Another relevant experiment that measured the global transcriptional response to stress was presented in ref (Causton et al., 2001). Their results indicate that glycolysis genes and ribosomal genes are a main part of the common environmental response genes (termed CER in ref (Causton et al., 2001)). The glycolysis genes expression was induced and the ribosomal genes expression was repressed after environmental perturbation that led to a stress response. This type of response was not observed in our experiment as these two groups were highly positively correlated along the expression dynamics in our data (see Fig. S3).

Table S2: Comparison to amino acid starvation response

AA starvation induced genes			
transitions	no3AT	40mM 3AT	629 genes
a	0.077652	-0.0038	
b	0.085632	0.084991	
c	-0.07792	-0.0038	
repressed			
transitions	no3AT	40mM 3AT	760 genes
a	0.108197	-0.01456	
b	0.099919	0.104144	
c	-0.06549	-0.028	
AA starvation GCN4 dependent induced genes			
transitions	no3AT	40mM 3AT	272 genes
a	-0.00389	-0.02827	
b	0.087263	0.040957	
c	-0.11973	0.117578	
repressed			368 genes
transitions	no3AT	40mM 3AT	
a	0.236281	0.019771	
b	0.183784	0.15525	
c	-0.17672	-0.03316	

AA starvation. Amino acid starvation expression data was derived from ref (Natarajan et al., 2001). The gene expression values that were changed significantly (at least 2 fold change, $p<0.05$) in their dataset C (+/- 100mM *3AT*) have been compared to our expression data for the induced and repressed genes. The table presents the correlation coefficients between the two experiments (ours and amino acid starvation). We compared the expression of genes induced or repressed in amino acid starvation to the expression changes in our experiments during 3 transitions as in table **S1** above (a-c). It is clear that there is no correlation between the pattern of expression observed in our experiments and the response to amino acid starvation, even for the high *3AT* experiment.

AA starvation GCN4 dependent. A sharper comparison to amino-acid starvation is possible by considering only the genes that are *Gcn4* dependent. Expression data was derived from ref (Natarajan et al., 2001). The gene expression values that were changed significantly (at least 2 fold change, $p < 0.05$) in their dataset C (+/- 100mM *3AT*) and were also changed significantly when comparing wild type to a *gcn4* mutant have been compared to our expression data for the 3 transients as above, for the induced and repressed genes. Again, there is no correlation between the expression patterns observed in our experiments and the *Gcn4*-dependent response.

References:

Causton, H.C., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S. and Young, R.A. (2001) Remodeling of yeast genome expression in response to environmental changes. *Molecular Biology of the Cell*, **12**, 323-337

.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., G., S., Eisen, M.B., Botstein, D. and Brown, P.O. (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell*, **11**, 4241-4257.

Kharchenko, P., Church, G.M. and Vitkup, D. (2005) Expression dynamics of a cellular metabolic network. *Molecular Systems Biology*, **1**, 1.

Natarajan, K., Meyer, M.R., Jackson, B.M., Slade, D., Roberts, C., Hinnebusch, A.G. and Marton, M.J. (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol. and Cellular Biologyl*, **21**, 4347-4368.

Shamir, R., Maron-Katz, A., A.Tanay, Linhart, C., Steinfeld, I., Sharan, R., Shiloh, Y. and Elkon, R. (200 (5EXPANDER – an integrative program suite for microarray data analysis. *BMC Bioinformatics*, **6**, 232.

Stolovicki, E., Dror, T., Brenner, N. and Braun, E. (2006) Synthetic gene recruitment reveals adaptive reprogramming of gene regulation in yeast. *Genetics* ,173 , 75-85.

Tamayo, P., Slonim, D., Mesirov, J., Zhu, Q., Kitareewan, S., Dmitrovsky, E., Lander, E.S. and Golub, T.R. (1999) Interpreting patterns of gene expression with self-organizing maps: Methods and application to hematopoietic differentiation. *Proc. Nat. Acad. Sci. USA*, **96**, 2907-2912.

www.yeastgenome.org.